AMENDMENTS TO THE CLAIMS

This listing replaces all prior versions and listings of claims in the application.

Listing of Claims

1-62. (Canceled)

63. (Currently Amended) An antibody or a functional fragment thereof, ~~of any one of claims 1 to 49~~ which binds to ~~TRAIL-R~~ TRAIL-R2 and induces apoptosis in carcinoma cells expressing TRAIL-R as a monomer independently of exogenous factors.

64. (Currently Amended) An antibody or a functional fragment thereof ~~of any one of claims 1 to 49~~, which binds to ~~TRAIL-R~~ TRAIL-R2 and induces apoptosis in carcinoma cells expressing TRAIL-R as a monomer independently of exogenous factors, and the survival rate of carcinoma cells in the following test using the said antibody or functional fragment thereof is 80% or less,

(1) Preparing Colo205 cells (ATCC No.CCL-222) which were colon carcinoma cells, at a concentration of 1.0 x 10^5/ml in RPMI-1640 medium containing 10% FCS, adding the cells to each well of a 96-well flat-bottomed plate at 100μl/well and culturing at 37°C under 5.0% carbon dioxide gas for 24 hours,

(2) Adding to each well of (1) an antibody or a functional fragment thereof which is bound to TRAIL-R dissolved in RPMI-1640 medium containing 10% FCS such that a concentration of the antibody or the functional fragment thereof becomes 1000ng/ml when it is added to each well at 10μl/well, culturing each well at 37°C under 5.0% carbon dioxide gas for 48 hours, washing each well once with PBS and adding a fresh RPMI-1640 medium containing 10% FCS at100μl/well,

(3) Adding 20 μl of MTS reagent (Cell Titer 96® AQueous Non-Radioactive Cell Proliferation Assay: Promega) to each well of (2) and culturing at 37°C under 5.0% carbon dioxide gas for 2 hours, and

(4) Measuring absorbance of each well of (3) at a wavelength of 490 nm (with a reference wavelength of 630 nm) using a microplate reader and calculating the survival rate of the cells using the reducibility of the mitochondria as an indicator,

wherein the survival rate of the cells is calculates using the following formula,

Survival rate (%) = 100 × (a-b)/(c-b) (wherein "a" represents the measured value of a well tested, "b" represents the measured value of a carcinomacell-free well, and "c" represents (i) the measured value of a well containing carcinomacells and a control antibody which is not bound to carcinoma cells and has the same subclass with the antibody or the functional fragment thereof bound to TRAIL-R when the antibody or the functional fragment thereof has a constant region, or (ii) the measured value of a well containing carcinoma cells and a control antibody which is not bound to the carcinoma cells and does not have a constant region when the antibody or the functional fragment thereof does not have a constant region).

65. (Original) An antibody or a functional fragment thereof of claim 64, wherein the survival rate is 60% or less.

66. (Original) An antibody or a functional fragment thereof of claim 64, wherein the survival rate is 40% or less.

67. (Original) An antibody or a functional fragment thereof of claim 64, wherein the survival rate is 20% or less.

68. (Original) An antibody or a functional fragment thereof of claim 64, wherein the survival rate is 10% or less.

69. (Currently Amended) An antibody ~~of any one of claims 1 to 49~~, which binds to ~~TRAIL-R~~ TRAIL-R2 and induces apoptosis in carcinoma cells expressing TRAIL-R as a monomer independently of exogenous factors, and the survival rate of carcinoma cells in the following test using the said antibody is 80% or less,

(1) Preparing Colo205 cells (ATCC No.CCL-222) which were colon carcinoma cells, at a concentration of 1.0 x 10^5/ml in RPMI-1640 medium containing 10% FCS, adding the cells to each well of a 96-well flat-bottomed plate at 100μl/well and culturing at 37°C under 5.0% carbon dioxide gas for 24 hours,

(2) Adding to each well of (1) an antibody which is bound to TRAIL-R dissolved in RPMI-1640 medium containing 10% FCS such that a concentration of the antibody becomes 1000ng/ml when it is added to each well at 10μl/well, culturing each well at 37°C under 5.0% carbon dioxide gas for 48 hours, washing each well once with PBS and adding a fresh RPMI-1640 medium containing 10% FCS at 100μl/well,

(3) Adding 20 μl of MTS reagent (Cell Titer 96® AQ$_{UEOUS}$ Non-Radioactive Cell Proliferation Assay: Promega) to each well of (2) and culturing at 37°C under 5.0% carbon dioxide gas for 2 hours, and

(4) Measuring absorbance of each well of (3) at a wavelength of 490 nm (with a reference wavelength of 630 nm) using a microplate reader and calculating the survival rate of the cells using the reducibility of the mitochondria as an indicator,

wherein the survival rate of the cells is calculates using the following formula,

Survival rate (%) = 100 × (a-b)/(c-b) (wherein "a" represents the measured value of a well tested, "b" represents the measured value of a carcinomacell-free well, and "c" represents the measured value of a well containing carcinoma cells and a control antibody which has the same subclass with the antibody bound to TRAIL-R and is not bound to the carcinoma cells).

70. (Original) An antibody of claim 69, wherein the survival rate is 60% or less.

71. (Original) An antibody of claim 69, wherein the survival rate is 40% or less.

72. (Original) An antibody of claim 69, wherein the survival rate is 20% or less.

73. (Original) An antibody of claim 69, wherein the survival rate is 10% or less.

74. (Currently Amended) An antibody ~~of any one of claims 1 to 49~~ which binds to ~~TRAIL-R~~ TRAIL-R2, and induces apoptosis in carcinoma cells expressing TRAIL-R as a monomer independently of exogenous factors, and the survival rate of carcinoma cells in the following test using the said antibody is 80% or less,

(1) Preparing Colo205 cells (ATCC No.CCL-222) which were colon carcinoma cells, at a concentration of 5 x 10^4/ml in RPMI-1640 medium containing 10% FCS, adding the cells to each well of a 96-well flat-bottomed plate at 100μl/well and culturing at 37°C under 5.0% carbon dioxide gas for 24 hours,

(2) Adding to each well of (1) an antibody which is bound to TRAIL-R dissolved in RPMI-1640 medium containing 10% FCS such that a concentration of the antibody becomes 1000ng/ml when it is added to each well at 10μl/well, culturing at 37°C under 5.0% carbon dioxide gas for 1 hour, adding a control antibody which has the same subclass with the antibody bound to TRAIL-R and is not bound to carcinoma cells such that a concentration is 100μg/ml, adding goat anti-human IgG (γ)-specific polyclonal antibodies at a final concentration of 10μg/ml, culturing each well at 37°C under 5.0% carbon dioxide gas for 2 days, washing each well once with PBS and adding a fresh RPMI-1640 medium containing 10% FCS at 100μl/well,

(3) Adding 20 μl of MTS reagent (Cell Titer 96® AQueous Non-Radioactive Cell Proliferation Assay: Promega) to each well of (2) and culturing at 37°C under 5.0% carbon dioxide gas for 2 hours, and

(4) Measuring absorbance of each well of (3) at a wavelength of 490 nm (with a reference wavelength of 630 nm) using a microplate reader and calculating the survival rate of the cells using the reducibility of the mitochondria as an indicator,

wherein the survival rate of the cells is calculates using the following formula,

Survival rate (%) = 100 × (a-b)/(c-b) (wherein "a" represents the measured value of a well tested, "b" represents the measured value of a carcinoma cell-free well, and "c" represents the

measured value of a well containing carcinoma cells and a control antibody which has the same subclass with the antibody bound to TRAIL-R and is not bound to the carcinoma cells).

75. (Original) An antibody of claim 74, wherein the survival rate is 60% or less.

76. (Original) An antibody of claim 74, wherein the survival rate is 40% or less.

77. (Original) An antibody of claim 74, wherein the survival rate is 20% or less.

78. (Original) An antibody of claim 74, wherein the survival rate is 10% or less.

79. (Currently Amended) An antibody ~~of any one of claims 1 to 49,~~ which binds to ~~TRAIL-R~~ TRAIL-R2, and induces apoptosis in carcinoma cells expressing TRAIL-R as a monomer independently of exogenous factors, and the survival rate of carcinoma cells in the following test using the said antibody is 80% or less,

(1) Preparing Colo205 cells (ATCC No.CCL-222) which were colon carcinoma cells, at a concentration of 5 x 10^4/ml in RPMI-1640 medium containing 10% FCS, adding the cells to each well of a 96-well flat-bottomed plate at 100μl/well and culturing at 37°C under 5.0% carbon dioxide gas for 24 hours,

(2) Adding to each well of (1) an antibody which is bound to TRAIL-R dissolved in RPMI-1640 medium containing 10% FCS such that a concentration of the antibody becomes 1000ng/ml when it is added to each well at 10μl/well, culturing at 37°C under 5.0% carbon dioxide gas for 1 hour, adding a control antibody which has the same subclass with the antibody bound to TRAIL-R and is not bound to carcinoma cell such that a concentration is 3μg/ml, adding goat anti-human IgG (γ)-specific polyclonal antibodies at a final concentration of 10μg/ml, culturing each well at 37°C under 5.0% carbon dioxide gas for 2 days, washing each well once with PBS and adding a fresh RPMI-1640 medium containing 10% FCS at 100μl/well,

(3) Adding 20 μl of MTS reagent (Cell Titer 96® AQ$_{UEOUS}$ Non-Radioactive Cell Proliferation Assay: Promega) to each well of (2) and culturing at 37°C under 5.0% carbon dioxide gas for 2 hours, and

(4) Measuring absorbance of each well of (3) at a wavelength of 490 nm (with a reference wavelength of 630 nm) using a microplate reader and calculating the survival rate of the cells using the reducibility of the mitochondria as an indicator,

wherein the survival rate of the cells is calculates using the following formula,

Survival rate (%) = 100 × (a-b)/(c-b) (wherein "a" represents the measured value of a well tested, "b" represents the measured value of a carcinoma cell-free well, and "c" represents the measured value of a well containing carcinoma cells and a control antibody which has the same subclass with the antibody bound to TRAIL-R and is not bound to the carcinoma cells).

80. (Original) An antibody of claim 79, wherein the survival rate is 60% or less.

81. (Original) An antibody of claim 79, wherein the survival rate is 40% or less.

82. (Original) An antibody of claim 79, wherein the survival rate is 20% or less.

83. (Original) An antibody of claim 79, wherein the survival rate is 10% or less.

84. (Currently Amended) An antibody or a functional fragment thereof, ~~of any one of claims 1 to 49~~ which binds to ~~TRAIL-R~~ TRAIL-R2, and induces apoptosis in carcinoma cells expressing TRAIL-R as a monomer independently of exogenous factors, and the survival rate of carcinoma cells on condition that (1)1.0 x 10^5/ml of carcinoma cells and (2)1000ng/ml of the antibody or the functional fragment thereof are cultured at 37°C under 5.0% carbon dioxide gas for 48 hours is 80% or less,

85. (Original) An antibody or a functional fragment thereof of claim 84, wherein the survival rate is 60% or less.

86. (Original) An antibody or a functional fragment thereof of claim 84, wherein the survival rate is 40% or less.

87. (Original) An antibody or a functional fragment thereof of claim 84, wherein the survival rate is 20% or less.

88. (Original) An antibody or a functional fragment thereof of claim 84, wherein the survival rate is 10% or less.

89. (Currently Amended) An antibody or a functional fragment thereof, ~~of any one of claims 1 to 49~~ which binds to ~~TRAIL-R~~ TRAIL-R2, and induces apoptosis in carcinoma cells expressing TRAIL-R as a monomer independently of exogenous factors, and the survival rate of carcinoma cells on condition that (1)5 x 10^4/ml of carcinoma cells, (2)1000ng/ml of the antibody, (3)100μg/ml of a control antibody or a functional fragment thereof which has the same subclass with the antibody or the functional fragment thereof bound to TRAIL-R and is not bound to carcinoma cells and (4)an antibody which binds to both the antibody or the functional fragment thereof bound to TRAIL-R and the control antibody are cultured at 37°C under 5.0% carbon dioxide gas for 48 hours is 80% or less.

90. (Original) An antibody or a functional fragment thereof of claim 89, wherein the survival rate is 60% or less.

91. (Original) An antibody or a functional fragment thereof of claim 89, wherein the survival rate is 40% or less.

92. (Original) An antibody or a functional fragment thereof of claim 89, wherein the survival rate is 20% or less.

93. (Original) An antibody or a functional fragment thereof of claim 89, wherein the survival rate is 10% or less.

94. (Currently Amended) An antibody or a functional fragment thereof, ~~of any one of claims 1 to 49~~ which binds to ~~TRAIL-R~~ TRAIL-R2, and induces apoptosis in carcinoma cells expressing TRAIL-R as a monomer independently of exogenous factors, and the survival rate of carcinoma cells on condition that (1)5 x 10^4/ml of carcinoma cells, (2)1000ng/ml of the antibody, (3)3μg/ml of a control antibody or a functional fragment thereof which has the same subclass with the antibody or the functional fragment thereof bound to TRAIL-R and is not bound to carcinoma

cells and (4)an antibody which binds to both the antibody or the functional fragment thereof bound to TRAIL-R and the control antibody are cultured at 37°C under 5.0% carbon dioxide gas for 48 hours is 80% or less.

95. (Original) An antibody or a functional fragment thereof of claim 94, wherein the survival rate is 60% or less.

96. (Original) An antibody or a functional fragment thereof of claim 94, wherein the survival rate is 40% or less.

97. (Original) An antibody or a functional fragment thereof of claim 94, wherein the survival rate is 20% or less.

98. (Original) An antibody or a functional fragment thereof of claim 94, wherein the survival rate is 10% or less.

99. (Currently Amended) An antibody or a functional fragment thereof of ~~any one of claims 84 to 98~~ claim 84, wherein the carcinoma cell is Colo205.

100. (Currently Amended) An antibody or a functional fragment thereof which is bound to ~~TRAIL-R of any one of claims 1 to 49~~ TRAIL-R2, the activity to induce apoptosis of which antibody or a functional fragment thereof on carcinoma cells expressing TRAIL-R does not substantially change depending on the presence or absence of an antibody which is bound to a constant region of the said antibody which is bound to TRAIL-R.

101. (Currently Amended) An antibody or a functional fragment thereof which is bound to ~~TRAIL-R of any one of claims 1 to 49~~ TRAIL-R2, wherein the survival rate of carcinoma cells expressing TRAIL-R does not substantially change depending on the presence or absence of an antibody which is bound to a constant region of the said antibody which is bound to TRAIL-R.

102. (Original) A therapeutic composition, comprising as an active ingredient the antibody or the functional fragment thereof of any one of claims 63 to 101.

103. (Original) A prophylactic or therapeutic agent against tumors, comprising as an active ingredient the antibody or the functional fragment thereof of any one of claims 63 to 101.

104. (Original) A prophylactic or therapeutic agent against tumors of claim 103, wherein the tumor is any one tumor selected from the group consisting of colon cancer, colorectal cancer, lung cancer, breast cancer, brain tumor, malignant melanoma, renal cell carcinoma, bladder cancer, leukemia, lymphomas, T cell lymphomas, multiple myeloma, gastric cancer, pancreas cancer, cervical cancer, endometrial carcinoma, ovarian cancer, esophageal cancer, liver cancer, head and neck squamous cell carcinoma, cutaneous cancer, urinary tract carcinoma, prostate cancer, choriocarcinoma, pharyngeal cancer, laryngeal cancer, thecomatosis, androblastoma, endometrium hyperplasy, endometriosis, embryoma, fibrosarcoma, Kaposi's sarcoma, hemangioma, cavernous hemangioma, angioblastoma, retinoblastoma, astrocytoma, neurofibroma, oligodendroglioma, medulloblastoma, ganglioneuroblastoma, glioma, rhabdomyosarcoma, hamartoblastoma, osteogenic sarcoma, leiomyosarcoma, thyroid sarcoma, Wilms tumor and the like.

105-107. (Canceled).

108. (Original) A method of producing an antibody or a functional fragment thereof of any one of claims 63 to 101, which comprisies,

(i) a step of immunizing an animal with TRAIL-R or a fragment thereof having the antigenicity, cells expressing the TRAIL-R or a fragment thereof having the antigenicity, or a DNA containing the gene encoding all or a part of the extracellular domain of TRAIL-R,

(ii) a step of obtaining antibodies from the animal,

(iii) a step of evaluating the activity of the antibodies to induce apoptosis in carcinoma cells expressing TRAIL-R independently of exogenous factors,

(iv) a step of separating a monomer antibody from the antibody,

(v) a step of evaluating the activity to induce apoptosis of the said monomer antibody, and

(vi) a step of selecting a monomer antibody having the activity to induce apoptosis.

109. (New) The method of producing an antibody or a functional fragment thereof of claim 108, wherein step (v) include the following test to determine a survival rate of carcinoma cell using the antibody,

(1) Preparing Colo205 cells (ATCC No.CCL-222) which were colon carcinoma cells, at a concentration of 1.0 x 105/ml in RPMI-1640 medium containing 10% FCS, adding the cells to each well of a 96-well flat-bottomed plate at 100μl/well and culturing at 37°C under 5.0% carbon dioxide gas for 24 hours,

(2) Adding to each well of (1) an antibody or a functional fragment thereof which is bound to TRAIL-R dissolved in RPMI-1640 medium containing 10% FCS such that a concentration of the antibody or the functional fragment thereof becomes 1000ng/ml when it is added to each well at 10μl/well, culturing each well at 37°C under 5.0% carbon dioxide gas for 48 hours, washing each well once with PBS and adding a fresh RPMI-1640 medium containing 10% FCS at100μl/well,

(3) Adding 20 μl of MTS reagent (Cell Titer 96® AQUEOUS Non-Radioactive Cell Proliferation Assay: Promega) to each well of (2) and culturing at 37°C under 5.0% carbon dioxide gas for 2 hours, and

(4) Measuring absorbance of each well of (3) at a wavelength of 490 nm (with a reference wavelength of 630 nm) using a microplate reader and calculating the survival rate of the cells using the reducibility of the mitochondria as an indicator,

wherein the survival rate of the cells is calculates using the following formula,

Survival rate (%) = 100 × (a-b)/(c-b) (wherein "a" represents the measured value of a well tested, "b" represents the measured value of a carcinomacell-free well, and "c" represents (i) the measured value of a well containing carcinomacells and a control antibody which is not bound to carcinoma cells and has the same subclass with the antibody or the functional fragment thereof bound to TRAIL-R when the antibody or the functional fragment thereof has a constant region, or (ii) the measured value of a well containing carcinoma cells and a control antibody which is not bound to the

carcinoma cells and does not have a constant region when the antibody or the functional fragment thereof does not have a constant region),

and the antibody having the survival rate of 80% or less is selected.

110. (New) The method of producing an antibody or a functional fragment thereof of claim 108, wherein step (v) include the following test to determine a survival rate of carcinoma cell using the antibody,

(1) Preparing Colo205 cells (ATCC No.CCL-222) which were colon carcinoma cells, at a concentration of 1.0 x 105/ml in RPMI-1640 medium containing 10% FCS, adding the cells to each well of a 96-well flat-bottomed plate at 100μl/well and culturing at 37°C under 5.0% carbon dioxide gas for 24 hours,

(2) Adding to each well of (1) an antibody which is bound to TRAIL-R dissolved in RPMI-1640 medium containing 10% FCS such that a concentration of the antibody becomes 1000ng/ml when it is added to each well at 10μl/well, culturing each well at 37°C under 5.0% carbon dioxide gas for 48 hours, washing each well once with PBS and adding a fresh RPMI-1640 medium containing 10% FCS at 100μl/well,

(3) Adding 20 μl of MTS reagent (Cell Titer 96® AQUEOUS Non-Radioactive Cell Proliferation Assay: Promega) to each well of (2) and culturing at 37°C under 5.0% carbon dioxide gas for 2 hours, and

(4) Measuring absorbance of each well of (3) at a wavelength of 490 nm (with a reference wavelength of 630 nm) using a microplate reader and calculating the survival rate of the cells using the reducibility of the mitochondria as an indicator,

wherein the survival rate of the cells is calculates using the following formula,

Survival rate (%) = 100 × (a-b)/(c-b) (wherein "a" represents the measured value of a well tested, "b" represents the measured value of a carcinomacell-free well, and "c" represents the

measured value of a well containing carcinoma cells and a control antibody which has the same subclass with the antibody bound to TRAIL-R and is not bound to the carcinoma cells),

and the antibody having the survival rate of 80% or less is selected.

111. (New) The method of producing an antibody or a functional fragment thereof of claim 108, wherein step (v) include the following test to determine a survival rate of carcinoma cell using the antibody,

(1) Preparing Colo205 cells (ATCC No.CCL-222) which were colon carcinoma cells, at a concentration of 5 x 104/ml in RPMI-1640 medium containing 10% FCS, adding the cells to each well of a 96-well flat-bottomed plate at 100µl/well and culturing at 37°C under 5.0% carbon dioxide gas for 24 hours,

(2) Adding to each well of (1) an antibody which is bound to TRAIL-R dissolved in RPMI-1640 medium containing 10% FCS such that a concentration of the antibody becomes 1000ng/ml when it is added to each well at 10µl/well, culturing at 37°C under 5.0% carbon dioxide gas for 1 hour, adding a control antibody which has the same subclass with the antibody bound to TRAIL-R and is not bound to carcinoma cells such that a concentration is 100µg/ml, adding goat anti-human IgG (γ)-specific polyclonal antibodies at a final concentration of 10µg/ml, culturing each well at 37°C under 5.0% carbon dioxide gas for 2 days, washing each well once with PBS and adding a fresh RPMI-1640 medium containing 10% FCS at 100µl/well,

(3) Adding 20 µl of MTS reagent (Cell Titer 96® AQUEOUS Non-Radioactive Cell Proliferation Assay: Promega) to each well of (2) and culturing at 37°C under 5.0% carbon dioxide gas for 2 hours, and

(4) Measuring absorbance of each well of (3) at a wavelength of 490 nm (with a reference wavelength of 630 nm) using a microplate reader and calculating the survival rate of the cells using the reducibility of the mitochondria as an indicator,

wherein the survival rate of the cells is calculates using the following formula,

Survival rate (%) = 100 × (a-b)/(c-b) (wherein "a" represents the measured value of a well tested, "b" represents the measured value of a carcinoma cell-free well, and "c" represents the measured value of a well containing carcinoma cells and a control antibody which has the same subclass with the antibody bound to TRAIL-R and is not bound to the carcinoma cells),

and the antibody having the survival rate of 80% or less is selected.

112. (New) The method of producing an antibody or a functional fragment thereof of claim 108, wherein step (v) include the following test to determine a survival rate of carcinoma cell using the antibody,

(1) Preparing Colo205 cells (ATCC No.CCL-222) which were colon carcinoma cells, at a concentration of 5 x 104/ml in RPMI-1640 medium containing 10% FCS, adding the cells to each well of a 96-well flat-bottomed plate at 100μl/well and culturing at 37°C under 5.0% carbon dioxide gas for 24 hours,

(2) Adding to each well of (1) an antibody which is bound to TRAIL-R dissolved in RPMI-1640 medium containing 10% FCS such that a concentration of the antibody becomes 1000ng/ml when it is added to each well at 10μl/well, culturing at 37°C under 5.0% carbon dioxide gas for 1 hour, adding a control antibody which has the same subclass with the antibody bound to TRAIL-R and is not bound to carcinoma cell such that a concentration is 3μg/ml, adding goat anti-human IgG (γ)-specific polyclonal antibodies at a final concentration of 10μg/ml, culturing each well at 37°C under 5.0% carbon dioxide gas for 2 days, washing each well once with PBS and adding a fresh RPMI-1640 medium containing 10% FCS at 100μl/well,

(3) Adding 20 μl of MTS reagent (Cell Titer 96® AQUEOUS Non-Radioactive Cell Proliferation Assay: Promega) to each well of (2) and culturing at 37°C under 5.0% carbon dioxide gas for 2 hours, and

(4) Measuring absorbance of each well of (3) at a wavelength of 490 nm (with a reference wavelength of 630 nm) using a microplate reader and calculating the survival rate of the cells using the reducibility of the mitochondria as an indicator,

wherein the survival rate of the cells is calculates using the following formula,

Survival rate (%) = 100 × (a-b)/(c-b) (wherein "a" represents the measured value of a well tested, "b" represents the measured value of a carcinoma cell-free well, and "c" represents the measured value of a well containing carcinoma cells and a control antibody which has the same subclass with the antibody bound to TRAIL-R and is not bound to the carcinoma cells),

and the antibody having the survival rate of 80% or less is selected.